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# Growth of Bacteria in Soluble Oil Emulsions[1]

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Cutting fluids are used in machine shops as lubricants and coolants when metals are being ground or cut. When so used they serve to keep the machined parts cool to prevent warping, prolong the life of the tool points, wash away chips and prevent rusting of the finished work.

Two types of cutting fluids are generally used, depending upon the requirements of the work. They are the straight and the soluble oils. Straight oils are petroleum or fatty oils, or mixtures of the two, with or without chemical treatment or additives to enhance lubricity. Soluble oils are usually petroleum oils mixed with emulsifying agents such as soaps of petroleum sulfonate, rosin, tall oil or fatty oil. The straight oils are used without mixing or are mixed with other oils, while the soluble oils are usually mixed with varying quantities of water to form stable, milky emulsions.

Bacteria are not known to grow in straight oils, as

water is necessary for synthesis of cellular materials. However, they do grow in soluble or emulsifiable oils (Duffett et al., 1943; Lee and Chandler, 1941; Liberthson, 1945; Westveer, 1951) when these have been mixed with water. Growth in this medium is thought to be responsible for several undesirable occurrences such as obnoxious odors, discoloration of the emulsion, and an increase in acidity with resultant breaking of the emulsion. Also, some workers are of the opinion that these bacteria are responsible for dermatitis of workers in contact with the oils, although Schwartz (1941) does not support this viewpoint.

This report deals with: (1) methods of determining numbers of bacteria in soluble oil emulsions; and (2) growth of bacteria in soluble oil emulsions.

## MATERIALS AND METHODS

### Method for Determination of Bacterial Populations in Soluble Oil Emulsions

*Diluents.* Diluents investigated were distilled water, M/20 phosphate buffers at pH 7.0, 7.2, 7.4, 7.6, and 8.0; 0.85 per cent sodium chloride; and 0.85 per cent

sodium chloride in m/20; phosphate buffers at pH 7.0, 7.6, and 8.0. Diluents were investigated by adding to the diluent sufficient of a washed suspension of mixed cultures obtained from several industrial plants to give a concentration of approximately 250 bacteria per ml. At intervals of 1, 30, 60, and 90 min quadruplicate plates were prepared with 1 ml of suspension using nutrient agar (Difco) as a plating medium. In these experiments and also in all subsequent work, media were held at 45 C before pouring and all plates were incubated for 2 days at 30 C before colonies were counted on a Quebec Colony Counter.

Table 1 shows the results of a typical experiment. All diluents except those containing saline were found to have little toxicity for the suspended organisms. However, because buffer at pH 7.0 yielded most consistent results it was used in all subsequent work except where stated otherwise.

TABLE 1. *Per cent change of bacteria from mixture of soluble oil. emulsions when suspended in various diluents*

| DILUENT | BACTERIA PER ML AT 1 MIN | PER CENT CHANGE | | |
|---|---|---|---|---|
| | | 30 min | 60 min | 90 min |
| Distilled water | 160 | 13.7 | 5.0 | 1.6 |
| 0.85 per cent saline | 171 | −33.9 | −85.4 | −96.4 |
| Buffer, pH 7.0 | 165 | 3.0 | 1.2 | 2.4 |
| Buffer, pH 7.2 | 165 | −1.2 | −12.1 | 2.4 |
| Buffer, pH 7.4 | 211 | −0.9 | 6.2 | 3.8 |
| Buffer, pH 7.6 | 172 | −14.5 | −8.1 | 4.6 |
| Buffer, pH 7.8 | 157 | 5.7 | 19.1 | 9.5 |
| Buffer, pH 8.0 | 158 | 3.2 | 0.6 | −17.7 |
| Buffered saline, pH 7.0 | 77 | 118.2 | 106.6 | 62.4 |
| Buffered saline, pH 7.6 | 51 | 233.4 | 263.0 | 202.0 |
| Buffered saline, pH 8.0 | 89 | 28.1 | 74.2 | 76.4 |

*Media.* Media investigated were nutrient agar and nutrient oil agar (nutrient agar containing 1.0 per cent of soluble oil). When 9 samples of emulsion were plated with these media nutrient agar gave significantly higher counts than nutrient oil agar, probably because the soluble oil rendered the latter medium very opaque. All colonies fished from the nutrient oil agar to nutrient agar grew well, indicating that oil was unnecessary for growth. Similar results have been observed by Stone et al., (1942). In all subsequent work nutrient agar was used as a plating medium.

### Growth of Bacteria in Soluble Oil Emulsions

Bacteria grow readily in soluble oil emulsions used in industry. This work, however, is concerned with several hitherto unreported aspects of growth of bacteria in soluble oil emulsions as well as confirming some of the observations of Duffett et al., (1943).

*Bacterial populations in industrial emulsions.* Thirty-six samples of emulsions from machine shops in Michigan, Illinois, and Wisconsin were investigated by the previously described methods. Figure 1 shows that most of the samples contained between $10^6$ and $10^8$ bacteria per ml. These results are in agreement with the work of Duffett et al., (1943).

*Growth of bacteria in different soluble oil emulsions.* Growth of mixed flora was followed in small stationary Erlenmeyer flasks, in gallon volumes of emulsion circulated twice daily, and in industrial pits containing several thousand gallons. Thirteen different soluble oils were investigated in the laboratory and two in industry. In the laboratory the inoculum used was composed of
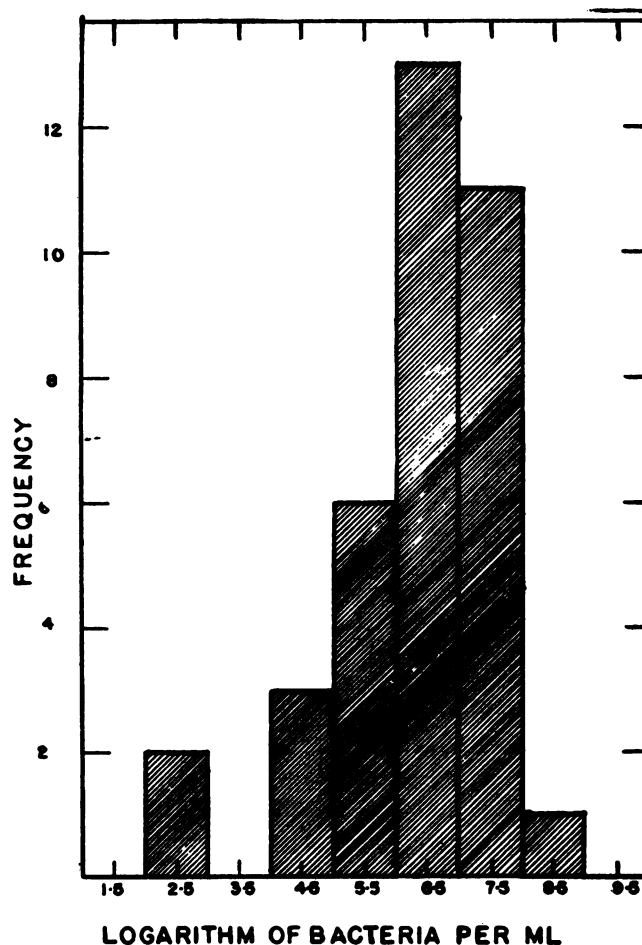


FIG. 1. Frequency distribution of bacterial populations in 36 samples of soluble oil emulsions obtained from industrial sources.

mixtures of emulsion samples taken from industrial sources and the medium was a 4 per cent emulsion of soluble oil in tap water with added iron chips. In the factory one of the pits (A) was filled with a 2 per cent emulsion, the other (B) with a translucent coolant which was not an emulsion. In these pits the inoculum was the residual indigenous flora.

Results of the laboratory experiments indicate that all 13 soluble oils supported microbial growth with populations between $10^7$ and $10^8$ cells per ml being obtained within two or three days. Figure 2 shows two

population curves typical of growth in the 13 soluble oils. Growth of the indigenous flora in fresh coolant in the machine shop is shown in table 2. Unfortunately a sample could not be obtained from system A at the time it was filled but it is obvious that the substrate
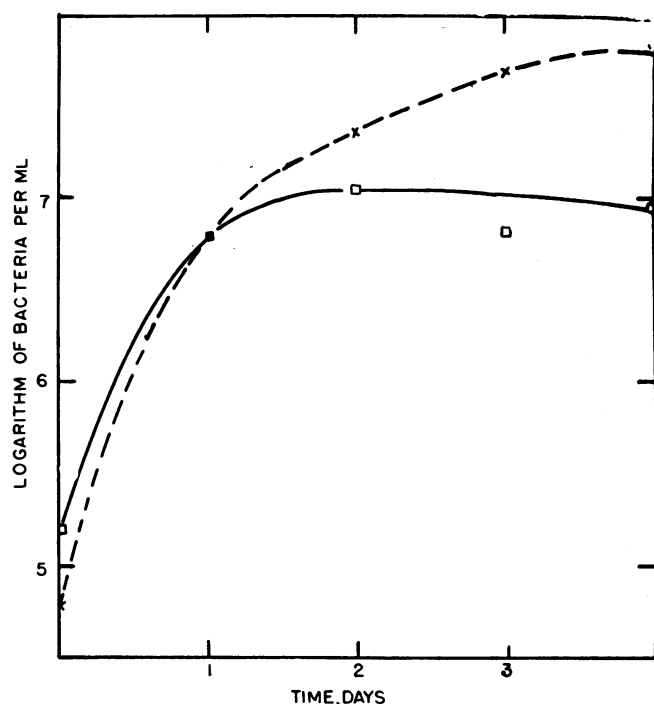


FIG. 2. Growth curves of mixed cultures in emulsions of two different soluble oils. These oils are representative of 13 oils studied.

TABLE 2. *Increase of bacterial populations in two cutting fluids in a machine shop*

| DAYS IN USE | BACTERIA PER ML | |
| --- | --- | --- |
| | A* | B† |
| 0 | | 200,000 |
| 1 | | |
| 2 | 16,000,000 | |
| 3 | | 270,000 |
| 4 | | |
| 5 | 46,000,000 | |
| 6 | | 12,300,000 |
| 7 | | |
| 8 | | |
| 9 | 91,000,000 | |
| 10 | | 9,000,000 |

\* Two per cent emulsion.
† Non-emulsified coolant.

in system A supported a much greater microbial population than did the non-emulsified coolant in system B.

*Growth of pure cultures in the laboratory.* Thirteen pure cultures isolated from three samples of emulsion obtained from industry were introduced into separate Erlenmeyer flasks containing sterile 4 per cent emulsion of a single soluble oil. All thirteen grew readily, as did

stock cultures of *Escherichia coli, Aerobacter aerogenes* and *Pseudomonas aeruginosa.* Figure 3 shows two growth curves typical of the 13 isolates studied. It can be seen that different levels of population were obtained by different cultures.

*Effect of oil concentration on microbial population in the emulsion.* The effect of the oil concentration was studied by inoculating emulsions containing 10.0, 1.0, 0.1, 0.01, and 0.0 per cent of soluble oil and following the growth curves at room temperature. The emulsion was made with tap water. The inoculum was a mixture (20 industrial samples) of organisms grown on nutrient agar containing 1 per cent soluble oil. The inoculum was washed 3 times in M/20 phosphate buffer at pH 7.0
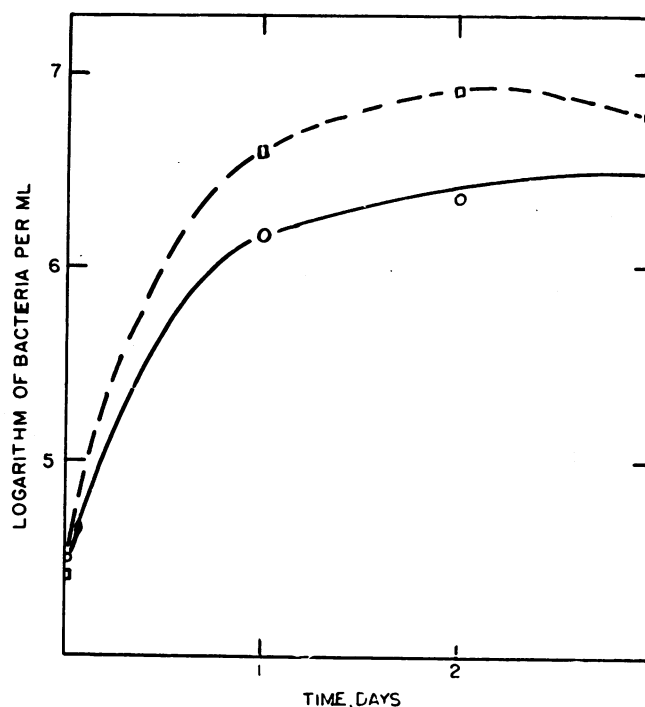


FIG. 3. Growth curves in soluble oil emulsions of two isolates obtained from industrially-used emulsions. These curves are representative of 13 isolates studied.

and diluted to give about $10^5$ cells per ml of inoculated emulsion. Figure 4 shows growth curves of the mixed inoculum in various concentrations of oil. Table 3 shows that as the oil concentration was increased the mean generation time in the logarithmic phase decreased. However, after 3 days there was a rapid decline of the microbial population in 0.1 per cent oil.

*Effect of added peptone on the microbial population in the emulsion.* Peptone, as a source of additional nitrogen for microbial growth, was added in concentrations of 0.5, 0.05, 0.005 and 0.0 per cent to a 4 per cent emulsion of soluble oil. Figure 5 shows that at 4 days the addition of 0.5 per cent peptone to the emulsion resulted in about a 100-fold increase in microbial population over that obtained in the absence of peptone. Replicate ex-

periments using the above as well as intermediate concentrations of peptone gave a linear curve for the microbial population at 4 days when log population was plotted against log peptone concentration. This curve fitted the formula $y = 1.47x$ for peptone concentrations from 0.005 to 0.25 per cent.

The addition of peptone to the emulsion resulted in the production of objectionable odors which were very pronounced in 0.5 per cent concentration of peptone but less so in 0.05 per cent.
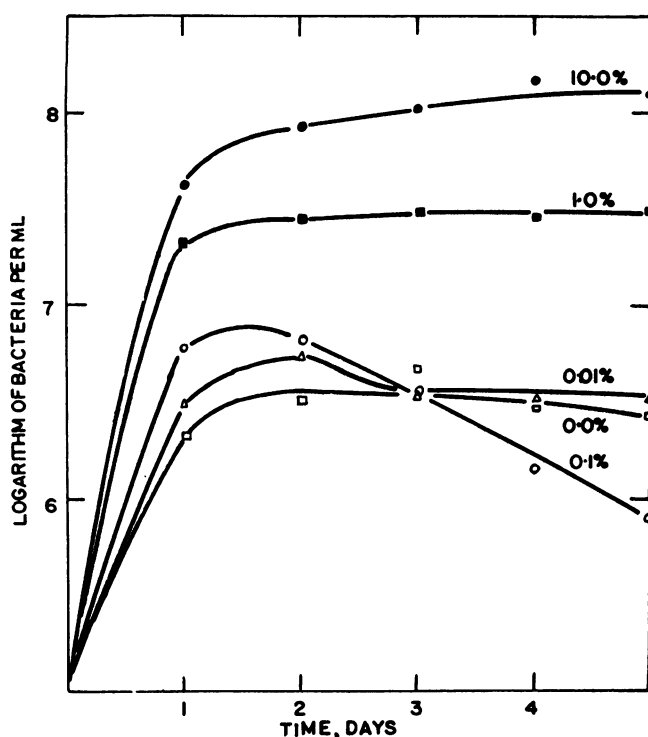


Fig. 4. Effect of oil concentration on the growth of bacteria in soluble oil emulsions.

TABLE 3. *Effect of oil concentration on the mean generation time of bacteria growing in soluble oil emulsions*

| PER CENT OIL | MEAN GENERATION TIME IN MINUTES |
|---|---|
| 10.00 | 173 |
| 1.00 | 193 |
| 0.10 | 247 |
| 0.01 | 289 |
| 0.00 | 367 |

*Possible sources of contamination of emulsions used in industry.* Several materials which might contain microorganisms capable of growing in soluble oil emulsions were used as inocula for a two per cent emulsion of soluble oil in tap water. Air, sputum, feces, sludge from a machine shop pit, sweepings from a machine shop floor and garden soil were investigated. In addition, an emulsion was made with river water. All emulsions were made in sterile equipment (Pivnick, 1952) and after inoculation were circulated continuously through iron chips by means of sterile compressed air. Plate

counts were made 45 min after inoculation and at intervals thereafter.
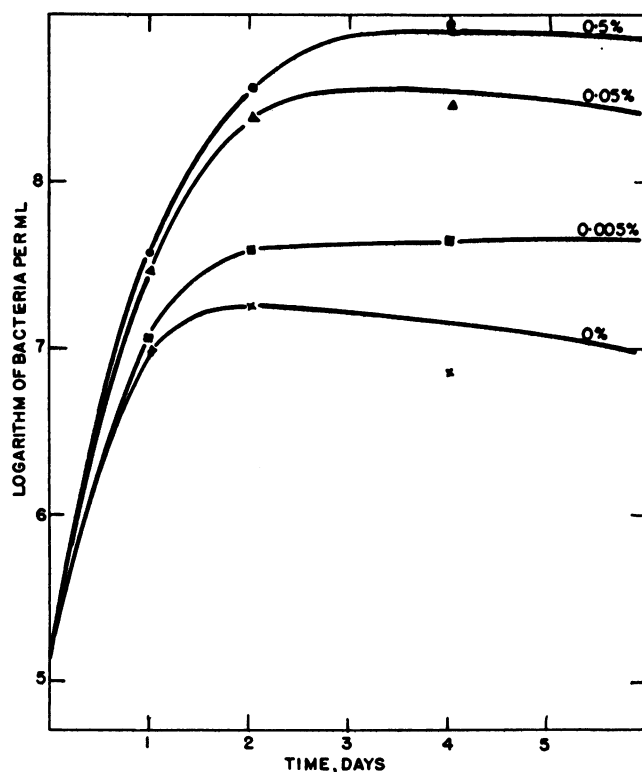


Fig. 5. Effect of added peptone on the growth of bacteria in soluble oil emulsions.
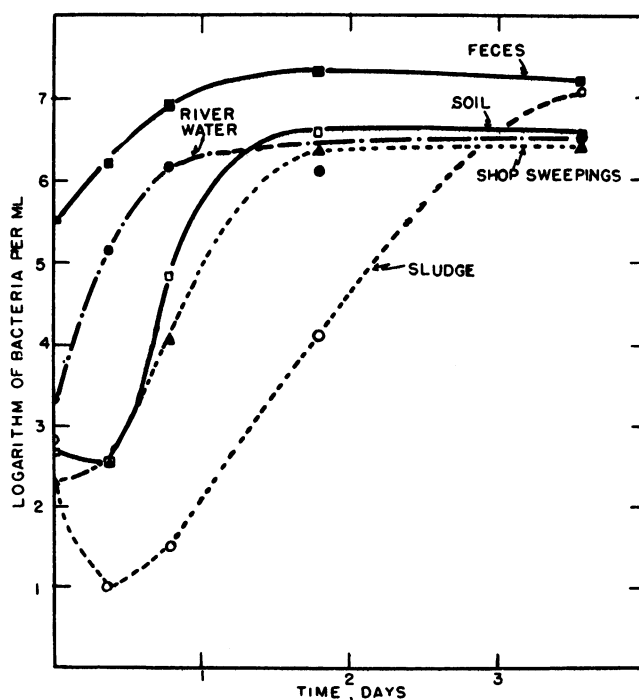


Fig. 6. Growth of contaminants in soluble oil emulsions

Figure 6 shows the growth of microorganisms resulting from inoculation with river water, feces, sludge, sweepings and garden soil. The results for sputum, aerial

contamination and uninoculated control are not graphed. The emulsion containing the sputum was sterile at 9 hr, the emulsion exposed to the air was sterile at 161 hr but contained 2,600,000 bacteria per ml at 353 hr, and the uninoculated tap water control remained sterile for the duration of the experiment.

## DISCUSSION

*Diluents and media.* Many of the diluents investigated are suitable for this work. Phosphate buffers and distilled water are generally adequate, although some experiments indicated that a pH of the diluent which differs markedly from that of the emulsion being investigated may rapidly kill the bacteria. Saline is definitely toxic, as shown in table 1. However, buffered saline gave results totally unlike those given by buffer alone or saline alone. Although all diluents received identical inocula, the mean count at one minute in diluents 1 to 8 inclusive (table 1) was 170 per ml, while in diluents 9 to 11 inclusive (buffered salines) the mean count was 72. This indicated that buffered salines caused a rapid decrease in bacterial populations as determined by the plate count. The decrease was not due to a killing effect because at 30 minutes and thereafter the counts were considerably greater than at one minute. A possible explanation of this phenomenon is that buffered saline caused an immediate clumping, but that on standing the clumps of bacteria broke apart.

Phosphate had an antagonistic activity towards the toxic effect of sodium chloride (table 1). At 90 minutes the buffered salines (diluents 9, 10, and 11) had a mean count of 145 bacteria per ml and distilled water contained 161 bacteria per ml; but saline contained only 6 bacteria per ml.

*Growth.* Bacterial populations in emulsions used in industry vary considerably but large populations comparable to those found in milk, fish, soil, etc., are rarely encountered. Very low microbial populations ($10^3$ to $10^4$ cells per ml) in industrial samples probably indicate the presence of disinfectants since 13 oils examined in the laboratory all supported growth of at least $10^7$ bacteria per ml, while some contained almost $10^8$ bacteria per ml. The level of population maintained may be a function of the nitrogen content of the emulsion because nitrogen determinations of several oils indicated that the greatest populations were obtained in those oils containing the most kjeldahl nitrogen. Increased growth upon the addition of peptone adds some weight to this hypothesis, although the carbon content of the peptone cannot be disregarded. It is unlikely that growth factors are involved because the washed inoculum grew well in emulsions made with tap water.

The increased numbers of bacteria produced upon increasing the oil concentration from 1.0 to 10.0 per cent indicates that some component of the oils is present in a relatively small amount. It is unlikely that carbon in the 1 per cent emulsion would be a limiting nutrient, much less in the 10 per cent emulsion. This again sug-gests that lack of nitrogenous compounds may be a cause of limited growth. The apparently anomalous growth curve for 0.1 per cent oil emulsion, which shows a much lower level of population after 4 days than was found in 0.01 or 0.0 per cent emulsion, is possibly the result of some autoantibiotic effect which was buffered by higher concentrations of oil and not produced in lower concentrations. This phenomenon was encountered in replicate experiments.

The growth from various types of inocula such as river water, feces, garden soil, etc., indicates the difficulty or even the futility of attempting to prevent microbial contamination. The failure of microorganisms to grow from the emulsion inoculated with sputum and the toxic effect of oil on pathogenic streptococci (Pivnick, 1952) are of some interest when one considers the amount of expectoration often directed into the cutting fluids. However, an effective control appears to be the addition of disinfectants, some of which have been studied recently (Pivnick and Fabian, 1953).

## ACKNOWLEDGEMENT

## SUMMARY

In the enumeration of bacteria in soluble oil emulsions, m/20 phosphate buffers at pH 7.0 to 8.0, or distilled water appear adequate as a diluent and nutrient agar as a plating medium. Diluents containing sodium chloride are undesirable. Bacteria grow readily in a variety of soluble oils, with soil, feces, river water, machine shop sweepings and pit sludge serving as inocula. Increasing the oil concentration of the emulsion decreases the mean generation time and increases the number of viable bacteria. The addition of peptone to the emulsion increases the number of viable bacteria and results in objectionable odors.

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